

(19)



Eur päisches Patentamt  
Eur pean Patent Office  
Offi ur péen des br vets



(11)

**EP 0 606 651 B1**

(12)

**EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention  
of the grant of the patent:  
07.07.1999 Bulletin 1999/27

(51) Int Cl.<sup>6</sup>: **C12M 1/20, B01L 3/00,  
C12M 3/06, C12M 1/12**

(21) Application number: **93121100.7**

(22) Date of filing: **30.12.1993**

(54) **Culture vessel**

Kulturgefäß

Réceptient de culture

(84) Designated Contracting States:  
**DE FR GB IT**

(30) Priority: **13.01.1993 US 3774**

(43) Date of publication of application:  
**20.07.1994 Bulletin 1994/29**

(73) Proprietor: **Becton, Dickinson and Company**  
**Franklin Lakes, New Jersey 07417-1880 (US)**

(72) Inventors:  
• **Stevens, Timothy A.**  
**Madison, New Jersey 07940 (US)**  
• **Mussi, Edward F.**  
**Hewitt, New Jersey 07421 (US)**

• **Henderson, Douglas P.**  
**Morristown, New Jersey 07960 (US)**  
• **Tyndorf, Tadeusz A.**  
**Manalpan Township, New Jersey 07726 (US)**

(74) Representative: **Selting, Günther, Dipl.-Ing. et al**  
**Patentanwälte**  
**von Kreisler-Selting-Werner,**  
**Bahnhofsvorplatz 1 (Deichmannhaus)**  
**50667 Köln (DE)**

(56) References cited:  
**EP-A- 0 183 973**                      **EP-A- 0 239 697**  
**EP-A- 0 495 213**                      **EP-A- 0 590 485**  
**EP-A- 0 590 513**                      **WO-A-92/07063**  
**US-A- 4 871 674**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

**EP 0 606 651 B1**

## Description

[0001] This invention relates to an apparatus useful for procedures in growing cells or tissue culture in vitro and more particularly for supporting, positioning and/or centering cell culture inserts that are used in the procedures.

[0002] Culture vessels are described in U.S. Patent No. 4,495,289 and 5,026,649 and European Patent Application No. 0 483 620 A2. Culture vessels comprise wells which generally have a circular shape and size which permits the introduction therein of a cell culture insert having a membrane upon which cell attachment, growth and differentiation occur. The culture vessels containing the wells are typically rectangular and have a standard size in order to accommodate standard analytical apparatus.

[0003] Conventional cell culture inserts used in culture vessels are described in U.S. Patent Nos. 4,871,674 and 5,026,649. U.S. Patent No. 4,871,674 discloses a cell culture insert which comprises discontinuous projecting parts for hanging the cell culture insert on an upper circumferential part of the culture vessel. U.S. Patent No. 5,026,649 discloses a cell culture insert which comprises a projecting part for hanging the culture cell on an upper circumferential part of the culture vessel, and further includes openings in the sidewalls for exchanging gas and for pipeting sample.

[0004] EP-A-0 239 697 discloses an apparatus for growing tissue cultures in vitro, said apparatus comprising a culture vessel and a cell culture insert suspended in a well of the vessel. The insert comprises a circular flange resting on a supporting surface of the vessel. The insert comprises a permeable membrane forming the bottom surface thereof. The insert may be rotated with respect to the vessel. The insert comprises a cutout for introducing a pipette to the wall of the insert into the culture vessel.

[0005] Although there are a number of culture vessels and cell culture inserts commercially available and described in patent publications, it is believed that there are no culture vessels or cell culture inserts available that can be used together to support, position and/or center a cell culture insert without interfering with the growing of tissue cultures in vitro.

[0006] It is an object of the present invention to provide an apparatus for use in growing tissue cultures in vitro wherein adjacent cell culture inserts are positioned in such a way that contamination is reduced.

[0007] The apparatus of the present invention is defined by claim 1.

[0008] The present invention is an apparatus for growing cells or tissue culture in vitro. The apparatus comprises a body with wells shaped to accommodate an insert, such as a cell culture insert.

[0009] The apparatus preferably comprises a test plate including an upper surface, a lower surface, and a plurality of wells. Each well is substantially disposed

between the upper and lower surface of the test plate. Each well comprises a sidewall, a bottom surface and a raised mouth surface. Most preferably, the raised mouth surface of each well includes a means for supporting a cell culture insert. This means is desirably raised from the upper surface.

[0010] The raised mouth surface preferably includes means for positioning and/or centering a cell culture insert. The preferred means is at least one integral lip portion extending from the raised mouth surface. The integral lip portion may be arranged to assist in positioning and/or centering a cell culture insert without limiting the movement of the cell culture insert.

[0011] The cell culture insert comprises an upper portion, a lower portion and sidewalls extending from the upper portion to the lower portion. The lower portion includes a bottom surface which may have attached thereto a flat permeable membrane. The upper portion includes a top surface which carries at least one outwardly extending flange. The flange allows the cell culture insert to be supported on the top surface of the well of the device so that there is clearance between the bottom of the membrane and the bottom surface of the well. The clearance provides a controlled static head and diffusion for the fluid in the device so that cells can be properly cultured.

[0012] The cell culture insert may further include at least one support footing on the bottom surface. The footing may provide a degree of clearance between the membrane and the bottom surface of the well.

[0013] Most preferably, the outer surface of the sidewall of the cell culture insert may further include means for restricting and/or preventing the flange of the cell culture insert from falling into the well of the device.

[0014] Although the apparatus of the present invention may be configured in a circle with one well, the preferable form of the apparatus is a multi-sided structure with wells in an ordered array of parallel rows. Furthermore, the integral lip portions on the raised mouth surface of each well may be positioned so that the integral lip portion of one well does not face an integral lip portion of an adjacent well. This positioning of the integral lip portions assures that the flanges of adjacent cell culture inserts positioned in the wells can be accessible, do not contact one another and that the cell culture insert may be aseptically placed and removed into or out of the well of the device.

[0015] The apparatus may further include a removable lid which can be positioned over the upper surface. The lid includes a top wall and a peripheral skirt that extends from the top wall. The lid serves to prevent the loss of sample from the well interior, to protect the contents of the device from the environment and to protect the user from the contents of the device should it contain a harmful or potentially harmful material.

[0016] The apparatus preferably receives a cell culture insert in the well. The cell culture insert is positioned into the well wherein the flanges of the cell culture insert

are supported on the raised mouth surface of the well and located between the integral lip portions. The integral lip portions assist in substantially positioning and/or centering the cell culture insert in the well and the raised mouth surface of the well substantially restricts the cell culture insert from falling into the well. Movement of the cell culture insert within the well may be such that the cell culture insert wall and the substantially parallel well sidewall can touch at one or more locations.

[0017] The exterior dimensions of the portion of the cell culture insert within the well of the apparatus are sufficiently less than the well diameter to allow a pipet or similar device to be positioned in the space between the well of the device and the cell culture insert for fluid filling or aspiration without disturbing or removing the cell culture insert from the well of the device. The space allows the pipet to reach the bottom of the well and introduce or remove medium from beneath the membrane and about the outer surface of the sidewall of the cell culture insert without contaminating the upper surface of the membrane.

[0018] The apparatus and related removable lid may be formed in different sizes and geometric configurations so as to be used with different size and geometric configured cell culture inserts. The removable lid may be formed to be positioned over the upper surface of the device in one orientation so as to reduce cross contamination between the wells in the event the lid is repositioned over the upper surface of the device. The apparatus and the removable lid are preferably made of an optically clear plastic to facilitate viewing of the wells and cell culture inserts.

[0019] An important feature of the present invention is that in a multi-well configuration, flange to flange contact between the cell culture inserts is prevented so as to reduce contamination. This is accomplished due to the orientation of the integral lip portions on each well wherein the integral lip portion of one well does not face or interfere with an integral lip portion of an adjacent well.

[0020] A further feature of the present invention is that the top surface configuration of the well of the apparatus may facilitate the aseptic placement and removal of the cell culture insert into or out of the well of the device with tweezers or forceps, as well as providing support to the cell culture insert when placed in the well of the apparatus.

### DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 is an exploded perspective view of a multi-well culture vessel and a cell culture insert.

[0022] FIG. 2 is a cross sectional view taken along lines 2-2 of FIG. 1 illustrating the means available for supporting, positioning and/or centering a cell culture insert in the well of a culture vessel.

[0023] FIG. 3 is an exploded perspective view of a multi-well culture vessel and the optional removal lid not

attached.

[0024] FIG. 4 is a top view of FIG. 1 illustrating the cell culture insert supported in the well of a culture vessel.

[0025] FIG. 5 is a cross-sectional view taken along lines 5-5 of FIG. 4 illustrating where a pipet tip, shown in phantom, may enter the space between the cell culture insert and the well of the culture vessel.

[0026] FIG. 6 is an exploded perspective view of an alternate embodiment of the present invention, a one-well culture vessel.

### DETAILED DESCRIPTION

[0027] An apparatus 10 for growing tissue cultures as shown in FIG. 1 includes a culture vessel 15 and a cell culture insert 40. Although a six well culture vessel is shown, it should be appreciated that the culture vessel may have one, eight, twelve, twenty-four or some other number of wells selected for the particular purpose for which the apparatus system is used.

[0028] As shown in FIGS. 1 and 2, culture vessel 15 includes a base 11 with an upper surface 12 and lower surface 13. The culture vessel further includes a number of wells 14 each comprising a sidewall 20 extending from the upper surface to the lower surface. A top portion 19 of the well comprises an open end 22, a top surface 24 extending from the upper surface and two integral lip portions 26 extending from the top surface. A bottom portion 18 of the well comprises a closed end 21.

[0029] Base 11, as illustrated in FIG. 1, typically is transparent and may be molded, for example, of polyvinylchloride or polystyrene. The culture vessel further includes sidewalls 27, 28, 29, 30, 31 and 32 that extend from upper surface 12 to lower surface 13. Sidewalls 27 and 29, and 28 and 31 are substantially parallel to each other respectively. Sidewall 30 is located between sidewalls 29 and 31 and sidewall 32 is located between sidewalls 31 and 27.

[0030] As shown in FIG. 1, the cell culture insert includes a body wall 42 having an outer wall surface 44 and an inner wall surface 46. Body wall 42 extends from an upper portion 48 to a lower portion 50. The body wall may taper from the upper portion to the lower portion. Upper portion 48 comprises a top surface 52 which carries two outwardly extending flanges 54. The flanges are located opposite from one another approximately 180° apart and are discontinuous. Flanges 54 comprise a horizontal orientation for resting on top surface 24 of well 14.

[0031] Lower portion 50 comprises a bottom surface 56 to which a microporous membrane 58 is adhered. The membrane may be made of suitable material including, but not limited to, perforated inert film, hydrated gel, or a layered combination.

[0032] In the embodiment illustrated, two projecting or extending tabs 60 are located on outer wall surface 44 of the cell culture insert. The tabs are located opposite from one another or approximately 180° apart and ap-

proximately half the distance between the upper and lower portions. The projecting tabs provide stability to the cell culture insert when it is in the well and shifted for pipette insertion. In the event the cell culture insert is shifted and one of the flanges falls into the well, the projecting tabs will substantially prevent the opposite flange from falling into the well.

[0033] The cell culture insert may further include feet or supports 61 located on the bottom surface. The feet are located opposite from one another or approximately 180° apart. Feet 61 are used when the extending flanges do not provide adequate support to the cell culture insert in a particular well configuration. It is most desirable that a space be maintained between the membrane on the bottom surface of the cell culture insert and the well of the culture vessel so that cells may be cultured on both sides of the membrane. Feet 61 further provide support to the cell culture insert when placed on a flat surface.

[0034] As shown in FIG. 3, the apparatus further includes a removable lid 70. Removable lid 70 includes a top wall 72, and peripheral sides 75, 76, 77, 78, 79 and 80 that extend from the top wall. The lid removably covers the upper surface of the culture vessel wherein the peripheral sides abut closely with the base of the culture vessel. In particular peripheral sides 77 and 79 of the lid abut with sidewalls 30 and 32 of the culture vessel. The lid is configured so that peripheral sides 77 and 79 are unable to abut with sidewalls 27, 28, 29 and 31 of the culture vessel. Therefore the lid may only be mated with the culture vessel in one way so that cross contamination is minimal between the wells of the culture vessel.

[0035] As shown in FIG. 4, wells 14 are sufficiently spaced from one another and the integral lip portions are oriented so as not to face or interfere with the integral lip portions of adjacent wells. The integral lip portions of each well are located opposite from one another or approximately 180° apart. This particular orientation of the integral lip portions substantially prevents flange to flange contact between the cell culture inserts and substantially reduces cross contamination between the cell culture inserts.

[0036] FIG. 5 illustrates the orientation of the cell culture insert as supported in culture vessel 15 wherein flange 54 is positioned on top surface 24 and between integral lip portions 26. Top surface 24 supports the cell culture insert and integral lip portions 26 assist in positioning and/or centering the cell culture insert without limiting movement of the cell culture insert. FIG. 5 also illustrates a pipet tip 64, in phantom, which enters the space 62 between outer wall surface 44 of the cell culture insert and sidewall 20 of the culture vessel.

[0037] The centering feature provided by integral lip portions 26 can be seen in Fig. 5. Cell culture insert 40 is set within well 14 and spaced sufficiently from well sidewall 20 as facilitated by top surface 24 and integral lip portions 26. Thus, capillary action should not occur to cause solution or media in space 62 from wicking up

outer surface 44 and entering the interior of the cell culture insert or spilling from well 14. The movement of the cell culture insert, however, may be such that the cell culture outer wall surface and the sidewall of the well can touch at one or more locations.

[0038] FIG. 6 illustrates a further embodiment of the present invention wherein the culture vessel includes one well. FIG. 6 includes many components which are substantially identical to the components of FIGS. 1-4. Accordingly, similar components performing similar functions will be numbered identically to those components of FIGS. 1-5, except that a suffix "a" will be used to identify those similar components in FIG. 6.

[0039] As illustrated in FIG. 6, a further embodiment of the invention includes a one well apparatus 90 having a culture vessel 100, a cell culture insert 40a and a removable lid 70a. The culture vessel includes a base 102 with an upper surface 104 and a lower surface 105. A well 106 comprising a sidewall 107 extends from the upper surface to the lower surface. A top portion 108 of the well comprises an open end 110, a top surface 112 extending from the upper surface and two integral lip portions 114 extending from the top surface. A lower portion 106 of the well comprises a closed end 118.

[0040] Base 102 typically is transparent and may be molded, for example, of polyethylene terephthalate. The culture vessel further includes sidewalls 120, 121, 122, 123, 124 and 125 that extend from upper surface 104 to lower surface 105.

[0041] As practitioners-in-the-art will understand, the culture vessel and cell culture insert of the present invention may be comprised of simple moldable parts which may be mass produced from a variety of materials, including, for example, polyethylene, polystyrene, polyethylene terephthalate, and polypropylene. As will be understood further by practitioners in the art, materials should be selected which provide a small degree of resiliency for the purpose of providing ease of insertion of the cell culture inserts into the culture vessel and ease of use for subsequent examination of the developed cultured cells.

## Claims

1. Apparatus for use in growing tissue cultures in vitro comprising:

a culture vessel (15) comprising an upper surface (12), a lower surface (13), a plurality of wells (14) extending from said upper surface (12) to said lower surface (13), a raised mouth surface (24) extending from said upper surface (12) of each well (14) for supporting a cell culture insert (40), and

a cell culture insert (40) suspended in one of said wells (14) and comprising an upper portion

(48), a lower portion (50), a sidewall (42) comprising an inner (46) and outer surface (44) and extending from said upper portion (48) to said lower portion (50), a permeable membrane (58) attached to said lower portion, a flange (54) extending from said outer surface of said sidewall for suspending said insert (40) in said one of said wells,

#### characterized in that

projecting portions (26) extend from said raised mouth surface (24) of each well for positioning a cell culture insert (40), and

said flange (54) is positioned on said raised mouth portion (24) between said projecting portions (26) to position said insert.

2. The apparatus of claim 1 wherein said projecting portions (26) comprise two projecting portions extending from said raised mouth surface (24) of each well (14) and being located opposite from one another.
3. The apparatus of claim 1 or 2 wherein said culture vessel (15) is substantially rectangular in shape and said wells (14) are in an ordered array of substantially parallel rows.
4. The apparatus of one of claims 1-3 wherein said flange (54) includes at least one discontinuous projecting flange extending from said upper portion (48).
5. The apparatus of one of claims 1-4 comprising at least one projecting tab (60) located on the outer surface (44) of said sidewall (42) and being constructed so as to provide access of sufficient size between the sidewall (42) of said insert (40) and the wall (20) of said one of said wells (14) of said culture vessel (15) so as to substantially prohibit capillary activity.
6. The apparatus of one of claims 1-5 wherein said flange (54) includes two discontinuous projecting flanges located opposite from one another and extending from said upper portion (48).
7. The apparatus of claim 5, characterized by two projecting tabs (60) located opposite one another on the outer surface of said sidewall (42) and between said upper portion (48) and said lower portion (50).
8. The apparatus of one of claims 1-6 wherein said projecting portions (26) of the wells (14) are in an ordered array to substantially prevent the flange (54) of one cell culture insert (40) from contacting

the flange of another cell culture insert.

#### Patentansprüche

1. Vorrichtung zur Verwendung für die in vitro-Züchtung von Gewebekulturen mit:

einem Kulturgefäß (15) mit einer oberen Fläche (12), einer unteren Fläche (13), einer Anzahl von Aufnahmebehältnissen (14), die sich von der oberen Fläche (12) zur unteren Fläche (13) erstrecken, einem erhabenen Öffnungsbereich (24), der von der oberen Fläche (12) jedes Aufnahmebehältnisses (14) absteht zur Aufnahme eines Zellkultureinsatzes (40), und

einem Zellkultureinsatz (40), der in eines der Aufnahmebehältnisse (14) eingehängt ist, mit einem Oberteil (48), einem Unterteil (50), einer Seitenwand (42), die eine Innenfläche (46) und eine Außenfläche (44) aufweist und sich vom oberen Bereich (48) zum unteren Bereich (50) erstreckt, einer permeablen Membran (58), die an dem Unterteil befestigt ist, einem Flansch (54), der von der Außenfläche der Seitenwand zum Einhängen des Einsatzes (40) in einen der Aufnahmebehältnisse absteht,

#### dadurch gekennzeichnet, daß

überstehende Bereiche (26) von dem Öffnungsbereich (24) jedes Aufnahmebehältnisses zur Positionierung eines Zellkultureinsatzes (40) abstehen, und

der Flansch (54) sich auf dem erhabenen Öffnungsbereich (24) zwischen den überstehenden Bereichen (26) zur Positionierung des Einsatzes befindet.

2. Vorrichtung gemäß Anspruch 1, bei dem die überstehenden Bereiche (26) aus zwei überstehenden Teilen bestehen, die über die erhabene Öffnungsfläche (24) jedes Aufnahmebehältnisses (14) verlaufen und einander gegenüberliegen.
3. Vorrichtung gemäß Anspruch 1 oder 2, bei der das Kulturgefäß (15) im wesentlichen von rechteckiger Form ist und die Aufnahmebehältnisse (14) im wesentlichen in parallelen Reihen angeordnet sind.
4. Vorrichtung gemäß einem der Ansprüche 1 - 3, bei der der Flansch (54) aus mindestens einem diskontinuierlichen überstehenden Flansch, der von dem oberen Bereich (48) absteht, gebildet wird.
5. Vorrichtung gemäß einem der Ansprüche 1 - 4, mit

mindestens einem abstehenden Vorsprung (60), der sich an der Außenfläche (44) der Seitenwand (42) befindet und so ausgebildet ist, daß ein ausreichend großer Abstand zwischen der Seitenwand (42) des Einsatzes (40) und der Wand (20) des Aufnahmebehältnisses (14) des Kulturgefäßes (15) verbleibt, daß Kapillaraktivität im wesentlichen verhindert wird.

6. Vorrichtung gemäß einem der Ansprüche 1 - 5, bei der der Flansch (54) aus zwei diskontinuierlichen, überstehenden Flanschen, die voneinander abgewandt verlaufen und von dem oberen Bereich (48) abste-  
hen, gebildet wird. 5
7. Vorrichtung gemäß Anspruch 5, die gekennzeichnet ist durch zwei abstehende Vorsprünge (60), die einander gegenüberliegend an der Außenfläche der Seitenwand (42) und zwischen dem oberen Be-  
reich (48) und dem unteren Bereich (50) angeord-  
net sind. 10
8. Vorrichtung gemäß einem der Ansprüche 1 - 6, bei der die überstehenden Bereiche (26) der Aufnahmebehältnisse (14) in einem geordneten Array so  
angeordnet sind, daß im wesentlichen verhindert wird, daß der Flansch (54) eines Zellkultureinsatzes (40) den Flansch eines anderen Zellkultureinsatzes  
berührt. 15

#### Revendications

1. Dispositif destiné à être utilisé dans la croissance de cultures de tissus in vitro comprenant : 20  
un récipient de culture (15) comprenant une surface supérieure (12), une surface inférieure (13), une pluralité de puits (14) s'étendant à partir de ladite surface supérieure (12) jusqu'à  
ladite surface inférieure (13), une surface d'em-  
bouchure élevée (24) s'étendant à partir de la-  
dite surface supérieure (12) de chaque puits  
(14) pour supporter un insert de culture de cel-  
lules (40), et un insert de culture de cellules (40)  
suspendu dans un desdits puits (14) et compren-  
nant une partie supérieure (48), une partie in-  
férieure (50), une paroi latérale (42) compren-  
nant une surface intérieure (46) et une surface  
extérieure (44) et s'étendant à partir de ladite  
partie supérieure (48) jusqu'à ladite partie in-  
férieure (50), une membrane perméable (58)  
fixée à ladite partie inférieure, un rebord (54)  
s'étendant depuis ladite surface extérieure de  
ladite paroi latérale pour suspendre ledit insert  
(40) dans l'un desdits puits, caractérisé en ce  
que des parties faisant saillie (26) se prolongent à partir de ladite surface d'embouchure 25

élevée (24) de chaque puits pour positionner un insert de culture de cellules (40), et ledit rebord (54) est positionné sur ladite partie d'embouchure élevée (24) entre lesdites parties en saillie (26) pour positionner ledit insert.

2. Dispositif selon la revendication 1, dans lequel lesdites parties en saillie (26) comprennent deux parties en saillie, s'étendant depuis ladite surface d'embouchure élevée (24) de chaque puits (14) et étant disposées opposées l'une à l'autre.
3. Dispositif selon la revendication 1 ou 2, dans lequel ledit récipient de culture (15) est essentiellement de forme rectangulaire et lesdits puits (14) sont selon un réseau ordonné de rangées essentiellement parallèles.
4. Dispositif selon l'une des revendications 1 à 3, dans lequel ledit rebord (54) comprend au moins un rebord faisant saillie discontinu s'étendant à partir de ladite partie supérieure (48).
5. Dispositif selon l'une des revendications 1 à 4, comprenant au moins une patte faisant saillie (60) située sur la surface extérieure (44) de ladite paroi latérale (42) et étant réalisée afin de fournir un accès d'une dimension suffisante entre la paroi latérale (42) dudit insert (40) et la paroi (20) de l'un desdits puits (14) dudit récipient de culture (15) de façon à empêcher essentiellement une activité capillaire.
6. Dispositif selon l'une des revendications 1 à 5, dans lequel ledit rebord (54) comprend deux rebords faisant saillie discontinus disposés à l'opposé l'un de l'autre et s'étendant à partir de ladite partie supérieure (48).
7. Dispositif selon la revendication 5, caractérisé par deux pattes faisant saillie (60) disposées à l'opposé l'une de l'autre sur la surface extérieure de ladite paroi latérale (42) et entre ladite partie supérieure (48) et ladite partie inférieure (50).
8. Dispositif selon l'une des revendications 1 à 6, dans lequel lesdites parties faisant saillie (26) des puits (14) sont selon un réseau ordonné pour empêcher essentiellement le rebord (54) d'un insert de culture de cellules (40) de venir au contact du rebord d'un autre insert de culture de cellules.

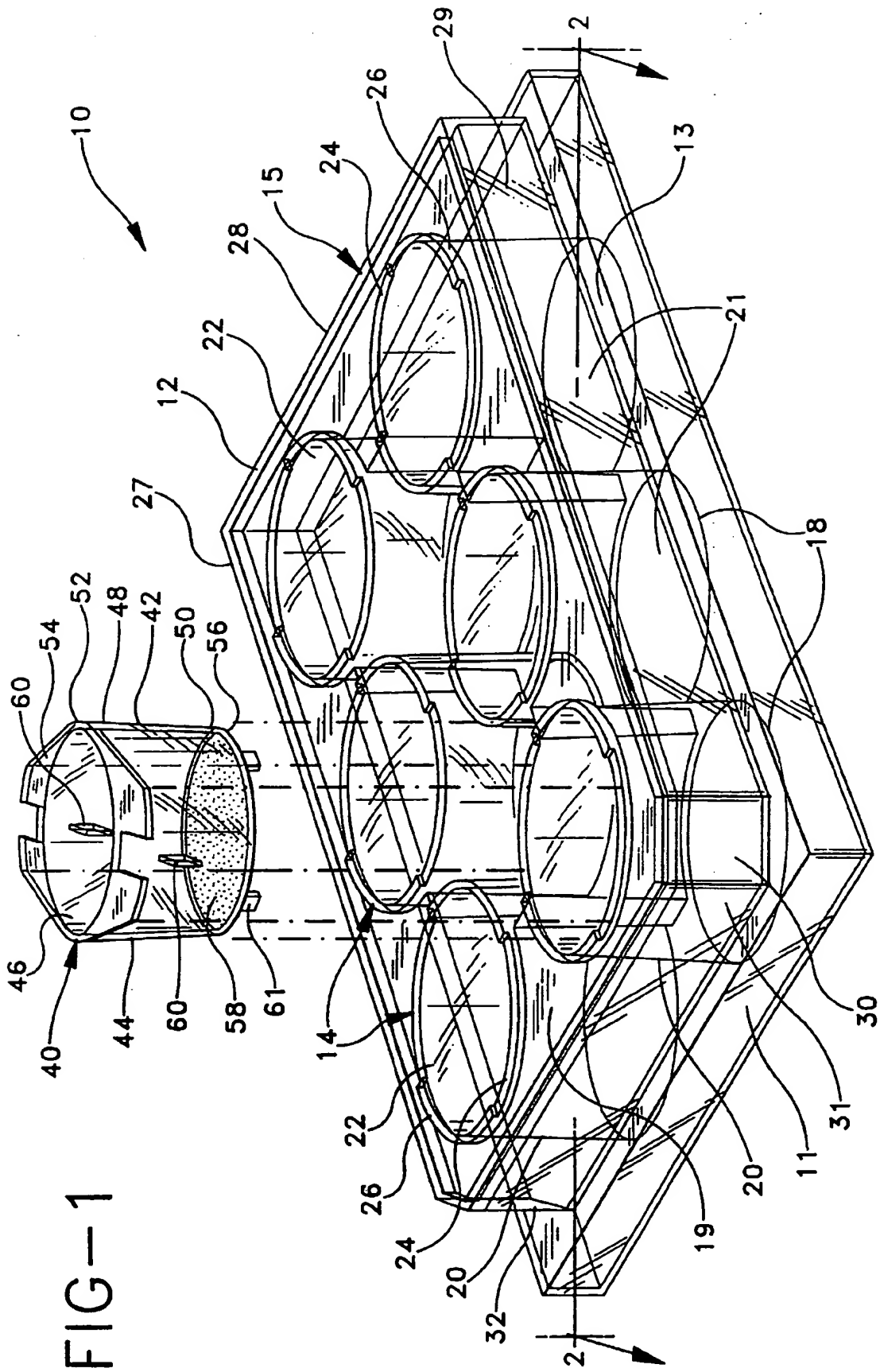


FIG-2

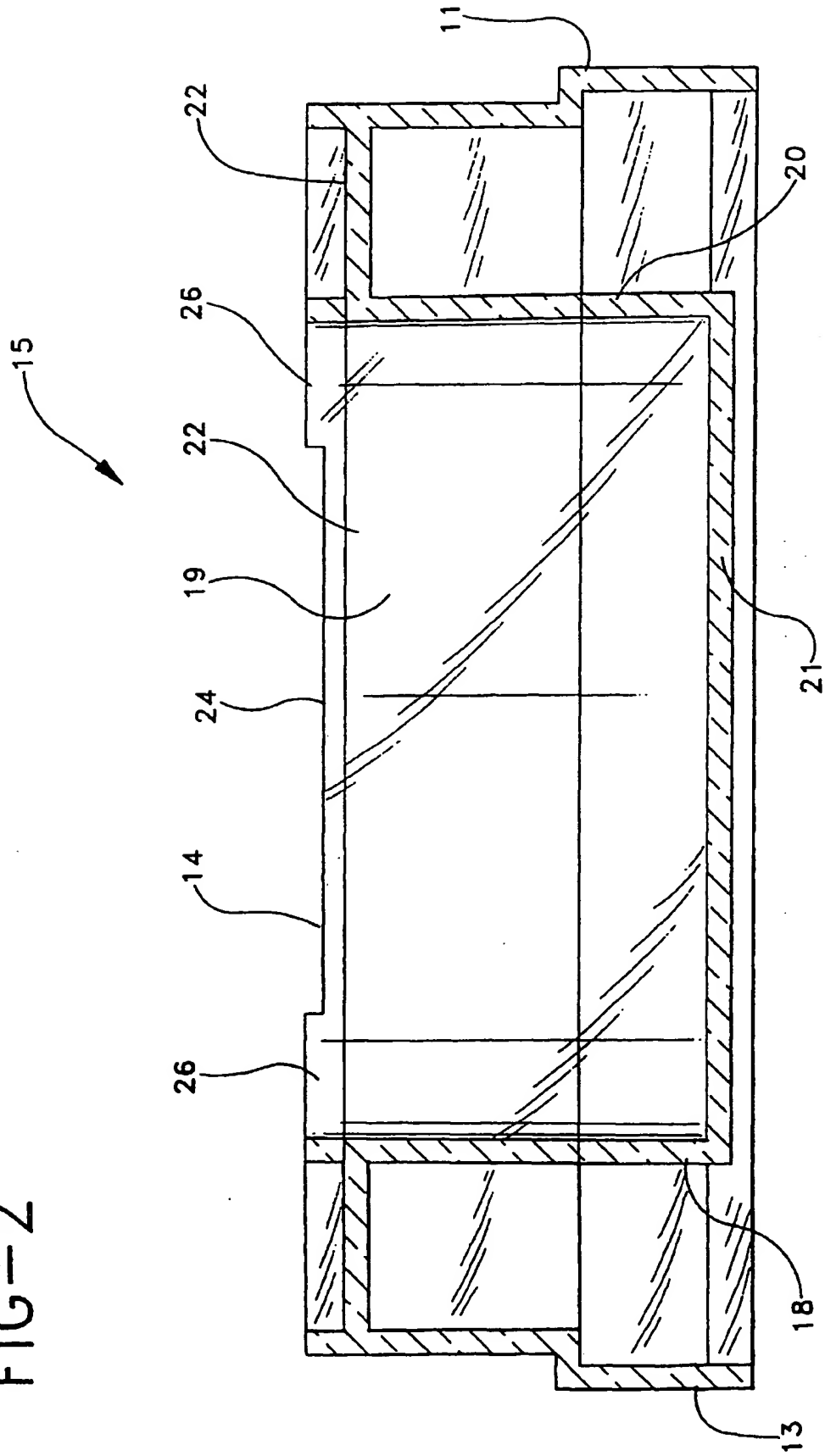
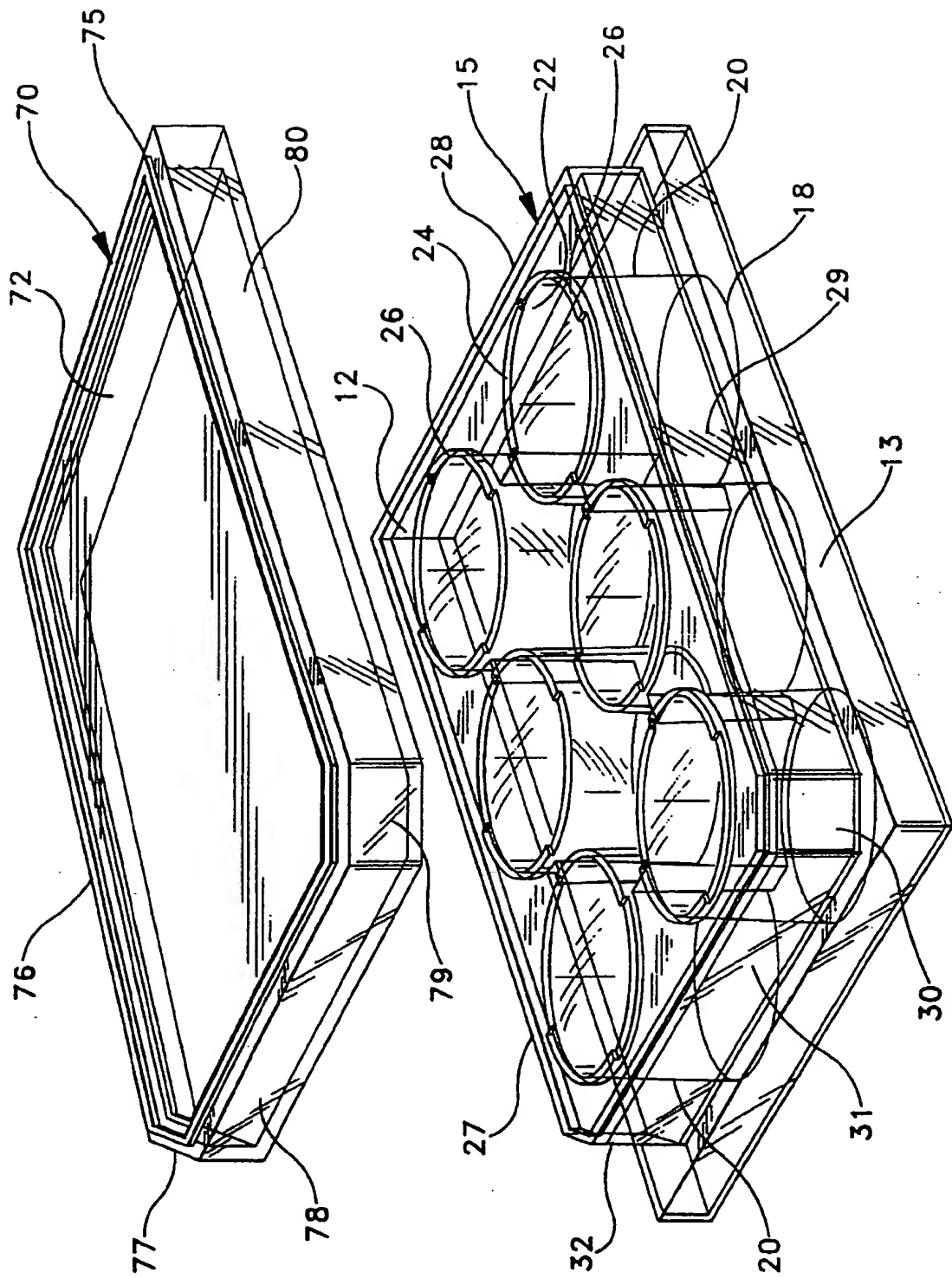




FIG-3



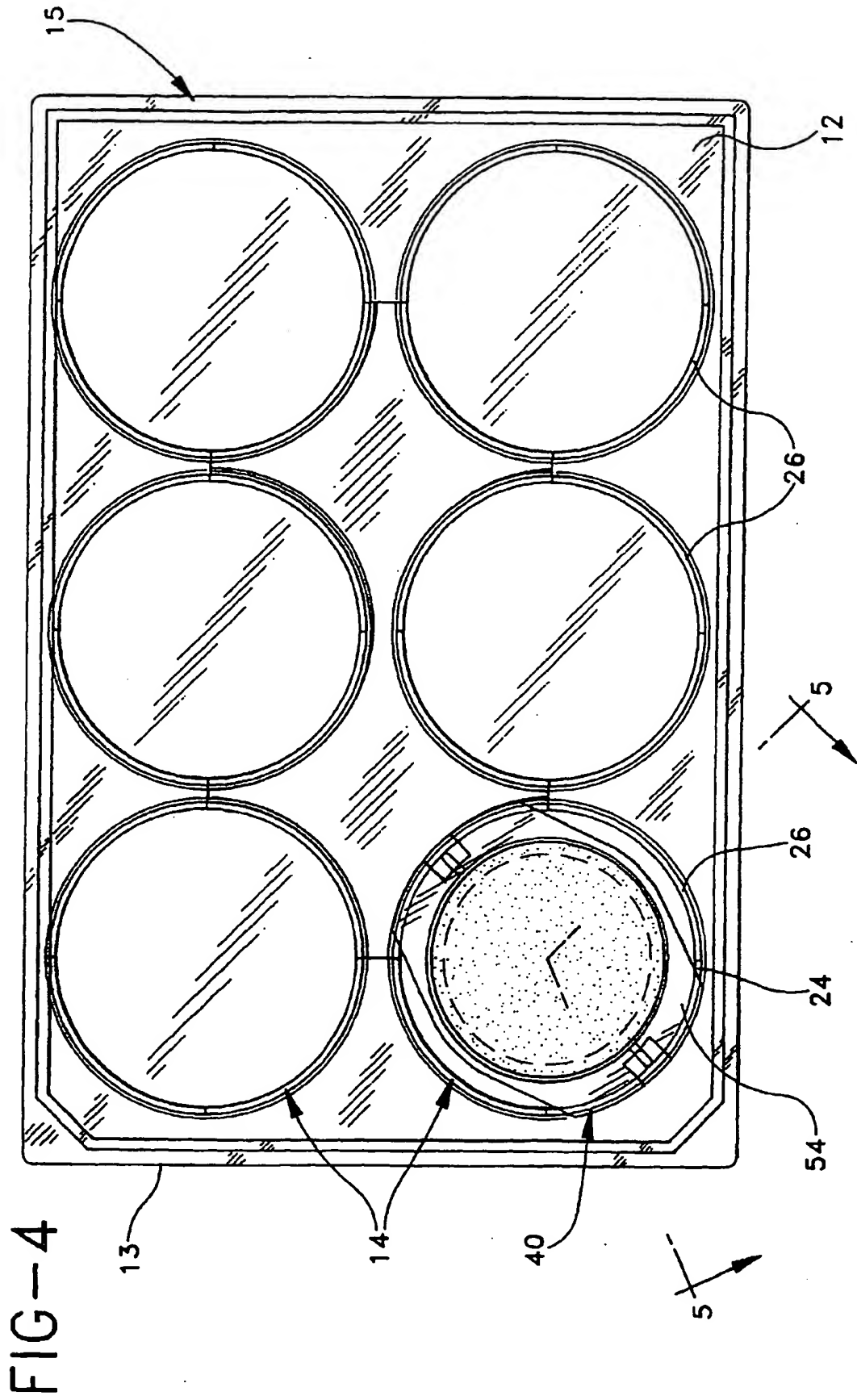


FIG-5

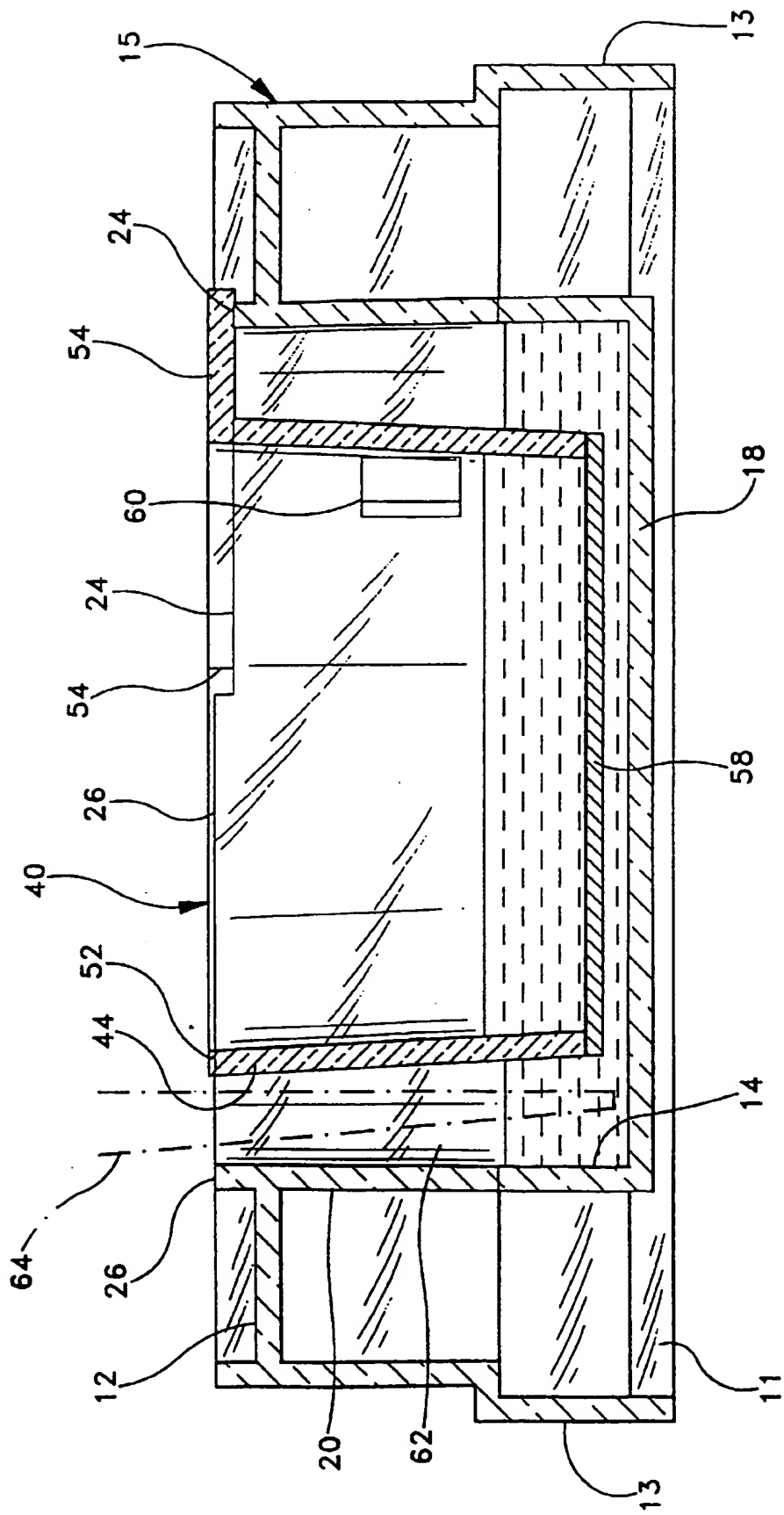


FIG-6

